

We claim:

1. A method for preparing a cell-line and/or tissue sample for *in situ* hybridization, comprising the steps of:
 - (a) pressure cooking the sample; and
 - (b) treating the pressure cooked sample with ammonia-ethanol and sodium borohydride.
2. The method of claim 1 wherein the sample is fixed-treated.
3. The method of claim 1 wherein the pressure cooking is performed in a decloaking chamber at a temperature of about 125 °C reaching a pressure of between about 20 to about 24 PSI
4. The method of claim 1 wherein the ammonia-ethanol is used in a concentration of about 0.25%.
5. The method of claim 1 wherein the sodium borohydrate is used in a concentration of about 5%.
6. The method of claim 1 wherein the fixed-treated sample is paraffin embedded.
7. The method of claim 1 wherein the sample is a tissue or a cell.
8. The method of claim 7 wherein the tissue or the cell is mammalian.
9. The method of claim 8 wherein the tissue or the cell is human.
10. A pressure cooked composition comprising:
 - (d) a fixed-treated tissue;
 - (e) ammonia-ethanol; and

(f) sodium borohydride.

11. The pressure cooked composition according to claim 10, wherein the fixed-treated tissue is paraffin embedded.

12. The pressure cooked composition of claim 10 wherein the ammonia-ethanol is used at a concentration of about 0.25%.

13. The pressure cooked composition of claim 10 wherein the sodium borohydride is used at a concentration of about 5%.

14. The use of the composition according to claim 10 in FISH.

15. The use according to claim 14 comprising a probe which comprises at least two fluorochromes detectable in quantitative computational fluorescence microscopic analysis.

16. The use according to claim 14 wherein an mRNA is hybridized with at least two different labeled probes.

17. The use according to claim 16 wherein the mRNA is a pre-mRNA.

18. The use according to claim 14 further comprising a quantification step wherein an mRNA expression level is calculated as a proportion of fluorochrome signal intensity of the mRNA.

19. The use according to claim 14 wherein the composition is spotted on a microarray.

20. A method of *in situ* hybridization for detecting and specifically identifying a plurality of transcription sites in a cell, the method comprising:

- (a) treating a sample with ammonia-ethanol and sodium borohydride;
- (b) pressure cooking the sample;
- (c) assigning a different barcode to more than one different target sequence, wherein each barcode comprises at least one fluorochrome, and at least one barcode comprises at least two different spectrally distinguishable fluorochromes;
- (d) providing a probe set specific to the more than one different target sequences containing a hybridization probe specific for each target nucleic acid, each hybridization probe comprising a single nucleic acid molecule complementary to the target sequence, wherein each probe is labeled with a fluorochrome and the fluorochromes in each probe set collectively identify the barcode for the target sequence of that probe set;
- (e) incubating the tissue with a set of probes specific for each of the at least two different target sequences such that each hybridization probe simultaneously hybridizes to the target sequence to which each hybridization probe is complementary; and
- (f) detecting the fluorochromes on the probe set hybridized to RNA transcribed from each target sequence, if present, thereby separately detecting and specifically identifying the plurality of transcripts, wherein the detecting includes spectrally distinguishing the different fluorochromes, wherein the fluorochromes on a detected transcript constitute a barcode for the detected transcript.

21. The method of claim 20 further comprising quantifying the fluorochromes by a computerized detection system.

22. The method of claim 20 wherein the cell has been pretreated with an agent.

22. The method of claim 20 wherein the fluorochromes are selected from the group consisting of Cy2, fluorX, Cy3, Cy3.5, Cy5, Cy5.5, Cy7, fluorescein and Texas Red.

23. The method of claim 22 wherein the probe is an oligonucleotide.
24. The method of claim 22 wherein the probe is a protein nucleic acid.
25. The method of claim 22 wherein the cell is part of a tissue sample.
26. The method of claim 25 wherein the tissue sample is derived from a mammal.
27. The method of claim 26 wherein the mammal is a human.
28. The use of the composition according to claim 14 in a toxicology analysis.
29. A method for identifying a potential therapeutic agent which modulates a level of a gene's expression in a tissue, the method comprising:
 - (a) preparing at least a first and second sample from at least a first and second tissue according to claim 1 wherein the samples are identical with the exception that the first tissue has been sampled from a cell-line, animal or human that has been treated with the agent whereas the second tissue has been sampled from a cell-line, animal, or human that has not been treated with the agent;
 - (b) detecting the level of the gene's transcription in the at least first and second tissue using FISH,wherein a difference in the detected levels indicates that the agent modulates the level of the gene's expression.
30. The method according to claim 29, wherein the modulation of the gene's expression is comprised in a gene pathway associated with therapeutic effects.
31. The method according to claim 29, wherein the modulation of the gene's expression is comprised in a gene pathway associated with toxic effects.

32. A method for reducing autofluorescence when performing FISH on a fixed treated tissue sample, the method comprising

- (a) pressure cooking the sample; and
- (b) treating the pressure cooked sample with ammonia-ethanol and sodium borohydride

wherein both steps are performed on the sample prior to performing FISH.